iGEM TU/e 2014
Biomedical Engineering

Eindhoven University of Technology
Room: Ceres 0.04
Den Dolech 2, 5612 AZ Eindhoven
The Netherlands
Tel. no. +31 50 247 55 59
2014.igem.org/Team:TU_Eindhoven

Date
4 August 2014

Protocol oil and water phase for bead encapsulation

This is a protocol for the production of the oil and water phase used in the microfluidic droplet device. It has been composed with the use of scientific resources and improved by trial and error during the experiments.

Technische Universiteit
Eindhoven
University of Technology

Table of contents

Title
Oil and Water Phase for bead encapsulation

1	**Oil Phase**	**3**
2	**Continuous phase**	**3**
2.1	**Bead encapsulation**	**3**
2.2	**Bacterial cell encapsulation**	**3**
3	**Density matching**	**3**
4	**References**	**4**

1 Oil Phase

- Fluorosurfactant 3.0% m/m in HFE-7500. This equals 4.1% v/v in HFE-7500.
- For 5 mL oil phase:
    - First add 4795 µL HFE-7500 to a 15 mL falcon tube.
    - Finally add 205 µL fluorosurfactant.

2 Continuous phase

2.1 Bead encapsulation

- For the first water phase a total volume of 1 mL is used:
    - First add 800 µL of demineralized water to a 15 mL falcon tube.
    - Then add 200 µL of the stock bead solution (=1.3*10^8 beads/mL).

- For the second water phase a total volume of 2 mL is used:
    - Add 2000 µL of demineralized water to a falcon tube.

2.2 Bacterial cell encapsulation

- For the first water phase a total volume of 1 mL is used:
    - Take 1 mL of the bacterial culture after protein expression and OD measurements are finished to a 15 mL falcon tube. (for protein expression and OD measurements see protocol *bacteria culturing for microfluidics*).
- For the second water phase a total volume of 1 mL is used:
    - Add 1 mL of PBS to a 15 mL falcon tube.

3 Density matching

- To match the density OptiPrep™ is used. The amount of OptiPrep™ that needs to be added can be calculated as follows:

D = $\frac{Vd+V1\ d1}{V+V1}$ with D = density of mixture; V = volume of OptiPrep™; d = density of OptiPrep™; V1 = volume of diluent; d1 = density of diluent.

(NOTE: Bead density is 1.05 g/mL; E. coli cell density is 1.16 g/mL).

- Calculate the amount of OptiPrep™ needed for both phases and add to the continuous phases.

(NOTE: both continuous phases need to have the same density so add OptiPrep™ to both continuous phases).

4 References

Mazutis, Linas, John Gilbert, W Lloyd Ung, David A Weitz, Andrew D Griffiths, and John A Heyman. "Single-cell analysis and sorting using droplet-based microfluidics." *Nature protocols* 8.5 (2013): 870-891. Print.
OptiPrep™ Preparation of gradient solutions. Biological Separations. ISSUE 2, 2009.